



## Field evaluation of mixed-seedlings with rice to alleviate flood stress for semi-arid cereals



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### ABSTRACT

Flash floods, erratically striking semi-arid regions, often cause field flooding and soil anoxia, resulting in crop losses on food staples, typically pearl millet (*Pennisetum glaucum* L.) and sorghum (*Sorghum bicolor* (L.) Moench). Recent glasshouse studies have indicated that rice (*Oryza* spp.) can enhance flood stress tolerance of co-growing dryland cereals by modifying their rhizosphere microenvironments via the oxygen released from its roots into the aqueous rhizosphere. We tested whether this phenomenon would be expressed under field flood conditions. The effects of mix-planting of pearl millet and sorghum with rice on their survival, growth and grain yields were evaluated under controlled field flooding in semi-arid Namibia during 2014/2015–2015/2016. Single-stand and mixed plant treatments were subjected to 11–22 day flood stress at the vegetative growth stage. Mixed planting increased plant survival rates in both pearl millet and sorghum. Grain yields of pearl millet and sorghum were reduced by flooding, in both the single-stand and mixed plant treatments, relative to the non-flooded upland yields, but the reduction was lower in the mixed plant treatments. In contrast, flooding increased rice yields. Both pearl millet–rice and sorghum–rice mixtures demonstrated higher land equivalent ratios, indicating a mixed planting advantage under flood conditions. These results indicate that mix-planting pearl millet and sorghum with rice could alleviate flood stress on dryland cereals. The results also suggest that with this cropping technique, rice could compensate for the dryland cereal yield losses due to field flooding.

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### 1. Introduction

Irregular floods increasingly strike semi-arid regions worldwide, often causing crop failures and hence food insecurity in the regions. In these regions, crop cultivation is dominated by drought-adapted cereals, such as pearl millet (*Pennisetum glaucum* L.) and sorghum (*Sorghum bicolor* L.) (Rai et al., 1999). Both crops are the staple food for most of the resource-poor smallholder farmers (Belton and Taylor, 2004). The global production area in 2014 was estimated at 31.1 million ha for pearl millet and 44.2 million ha for sorghum, with Africa constituting 63% and 65% of the crop areas,

respectively (FAO, 2015), but Sub-Saharan Africa has the highest proportion of food-insecure people (Porter et al., 2014). In semi-arid Sub-Saharan Africa, research on pearl millet and sorghum has been conducted mainly focusing on improving the genetic and physiological traits associated with drought tolerance, and the resultant genotypes have been distributed in various countries in the region (Ahmed et al., 2000; Mgonja et al., 2005). However, grain production in this region usually fails when high rainfall floods occur, because of the susceptibility to the flood stress of pearl millet (Sharma and Swarup, 1989; Zegada-Lizarazu and Iijima, 2005) and sorghum (Orchard and Jessop, 1984; Promkhambut et al., 2010, 2011). In Namibia, a semi-arid Sub-Saharan country in southwestern Africa, seasonal, high-rainfall floods have recently become a common occurrence, particularly in the country's main cropping areas of the populous northern region (Mendelsohn et al., 2013;

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**Table 1**  
Growth conditions during field experiments in 2014–2016.

Experimental period				Temperature (°C)			Relative humidity (%)			Solar radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	Rainfall (mm)
Year	Duration (from sowing to end of experiment)	Description	Flooding period (DAS) <sup>a</sup>	Mean	Max.	Min.	Mean	Max.	Min.		
2014/2015 a	18 Sep.–19 Jan.	Survival and production	21–43	27.0	34.6	19.5	42.2	66.5	23.3	25.7	127.2
2014/2015 b	30 Oct.–17 Mar.	Survival and production	21–36	26.9	34.2	19.7	45.4	70.9	24.7	26.4	185.7
2014/2015 c	30 Oct.–18 Dec.	Survival	21–36	24.3	30.5	18.4	63.9	86.7	40.8	22.4	69.9
2014/2015 d	13 Nov.–29 Dec.	Survival	21–32	27.3	35.1	18.9	26.8	48.7	12.6	29.1	0.6
2015/2016	17 Sep.–18 Jan.	Production and LER	21–36	28.5	36.1	20.3	38.4	62.2	21.5	33.2	113.0

The seedlings were transplanted to the field at 21 and 28 days after sowing of pearl millet (and/or sorghum) and rice, respectively. Weather data were collected starting from the flooding treatment to the end of the experiments. LER, Land Equivalent Ratio.

<sup>a</sup> Days After Sowing of pearl millet and/or sorghum; Max., maximum; Min., minimum.

Iijima, 2011; Suzuki et al., 2013; Mizuochi et al., 2014), causing losses in the yield of pearl millet and sorghum (Anthonj et al., 2015).

Soil flooding or waterlogging triggers a chain of reactions in the soil solution. These include the induction of hypoxia (sub-optimal oxygen [O<sub>2</sub>]) or anoxia (depletion of O<sub>2</sub>); elevation of carbon dioxide, methane and ethylene concentrations; reduction of aerobic and proliferation of anaerobic microbe populations and the accumulation of organic acids and reduced phytotoxins such as Fe<sup>2+</sup>, Mn<sup>2+</sup> and H<sub>2</sub>S in the soil solution (Colmer and Voesenek, 2009). Because O<sub>2</sub> is a fundamental requirement for plant growth, plants that do not possess mechanisms for flood tolerance, such as most dryland crops, experience poor growth and may even die because of flood stress (Setter and Belford, 1990). Rice (*Oryza* spp.) is a grain crop adapted to wetland environments, and the demand for rice in Sub-Saharan Africa has been increasing. Unlike most dryland crops, rice roots possess an efficient internal aeration system and release O<sub>2</sub> into the aqueous rhizosphere by radial O<sub>2</sub> loss, which are the mechanisms that allow rice roots to grow in flooded, anoxic soils (Joshi et al., 1973; Armstrong, 1979; Colmer, 2003; Kirk, 2003). In the rhizosphere, O<sub>2</sub> is used by soil microbes for respiration but also serves to re-oxidize reduced phytotoxins, mobilize nutrients and maintain aeration (Armstrong, 1979; Ando et al., 1983; Colmer, 2003; Kirk and Kronzucker, 2005), thus mitigating the unfavourable effects of soil flooding on plants (Armstrong and Armstrong, 2005).

Mixed cropping or intercropping generally induces competition for resources, but the co-growing plants can also improve the microclimate of their neighbours through rhizosphere interaction (Brooker, 2006; Brooker et al., 2015). Thus, under mixed cropping, both suppressive and supportive effects of plants on their neighbours occur simultaneously, and the net growth will be the outcome of these opposing effects (Maestre et al., 2003; Zhang and Li, 2003). One of the supportive or facilitative effects is the supply of nitrogen nutrition by legumes to non-leguminous crops such as cereals (Li et al., 1999; Xiao et al., 2004; Mucheru-Muna et al., 2010; Ramirez-Garcia et al., 2015).

Besides nutritional interactions, our previous study indicated that wetland-adapted plant species could alleviate the adverse effects of soil flooding on susceptible species (Iijima et al., 2016). Mix-planting pearl millet or sorghum with rice improved the photosynthetic rate, transpiration rate and biomass of co-growing pearl millet and sorghum seedlings grown under O<sub>2</sub>-deficient solution culture conditions. The mixed-seedling cropping technique could serve as one of the agronomic countermeasures to ensure constant staple grain production under flood conditions. However, the solution culture experiments could only demonstrate the possible scenario; therefore, there was a need to certify whether the phenomenon observed in the solution culture would be expressed in the field, where O<sub>2</sub> diffusion would be slower and microbial O<sub>2</sub> demand would be higher. In the present study, we assessed the effect of rice on the survival rate and grain production of companion pearl millet and sorghum subjected to field flooding at the

vegetative growth stage, and evaluated the productivity of the crop mixtures.

## 2. Materials and methods

### 2.1. Experimental sites

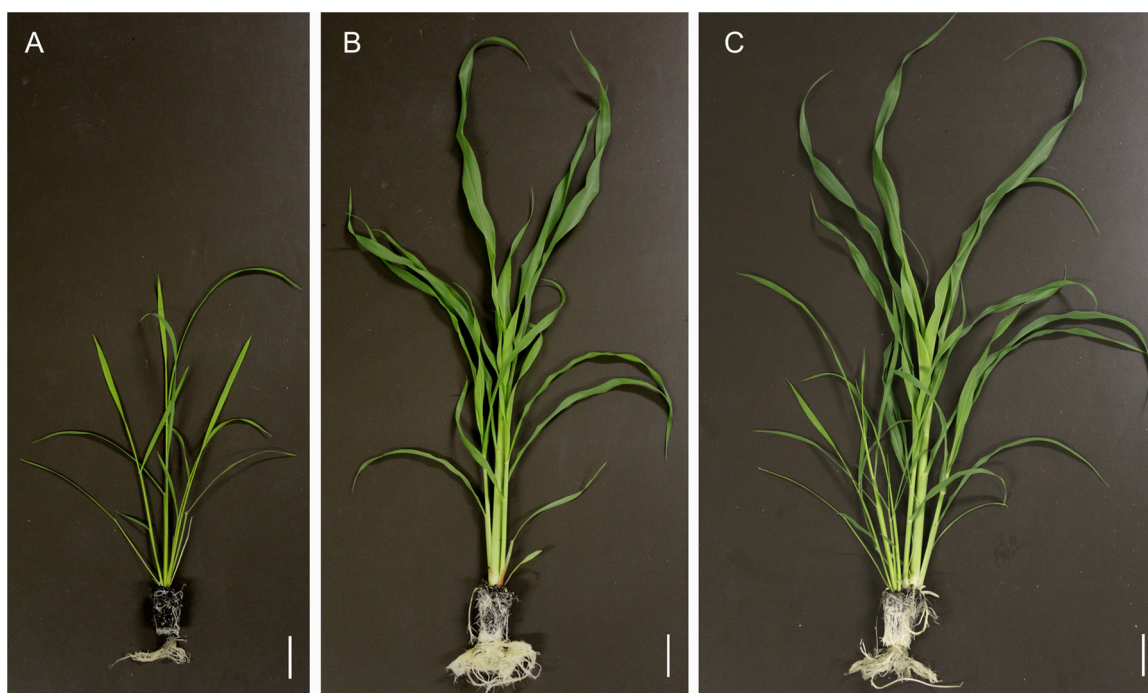
Mixed cropping experiments (Table 1) were conducted for 2 cropping seasons (2014/2015 and 2015/2016) at the University of Namibia Ogongo Campus (17°41'S, 15°18'E, 1109 m ASL), located in North-Central Namibia. North-Central Namibia has a semi-arid climate, annual mean temperature of >22 °C and annual average rainfall of 400–450 mm. This area is located in the Cuvelai drainage basin, originating in southern Angola where rainfall is higher. The basin is characterized by a huge network of seasonal wetlands (locally called *oshanas*), which irregularly overflow into local crop-lands owing to inflows from Angolan highlands or occasionally from localised high summer rainfall (Mendelsohn et al., 2013). During the study period, the weather data were collected using the Bowen ratio measuring system (C-AWS-BW3, Climatec, Japan) close to the experimental field. Growth periods and mean daily temperature, relative humidity, solar radiation and total rainfall for each experiment are demonstrated (Table 1). The topsoil (0–20 cm) at the experimental site in Namibia was classified as sand, with a texture of 93.5% sand, 2.0% clay and 4.5% silt, with 2.8 g total C kg<sup>-1</sup>, 0.28 g total N kg<sup>-1</sup>, 6.3 mg available P kg<sup>-1</sup>, 38.1 mg K kg<sup>-1</sup> and a pH (H<sub>2</sub>O) of 7.0.

### 2.2. Plant materials

In the present study, we used pearl millet (*Pennisetum glaucum* L. cv. Okashana 2) and sorghum (*Sorghum bicolor* (L.) Moench cv. Macia), adapted to semi-arid conditions, and rice (Interspecies of *Oryza. sativa* L. and *O. glaberrima* Steud. cv. NERICA4) as the flood-adapted crop. Okashana 2 and Macia, cultivated in several Southern African countries such as Namibia, were acquired from a Namibian seed company. NERICA4, an upland cultivar sourced from AfricaRice, Benin, West Africa, is promoted for cultivation among subsistence farmers in many Sub-Saharan African countries, such as Namibia.

### 2.3. The use of mixed-seedlings and seedling establishment

Seed pre-germination and sowing were performed as per the methods described previously (Iijima et al., 2016). The seedling mix of the wetland-adapted (rice) and dryland-adapted (pearl millet and sorghum) crop species (Fig. 1) was used in this experiment. This mixed-seedling system was intended to enhance the intertwining of the roots of the two species. It involved growing the mixed-seedlings in a small container, to allow the development of a dense root mat under the container. This was thought to contribute



**Fig. 1.** Growth of pearl millet-rice mixed seedling in cell tray. A, single-stand rice; B, single-stand pearl millet; and C, pearl millet mix-planted with rice. Scale bar (lower right in each figure) = 50 mm.

to efficient  $O_2$  transfer between the tangled roots of the two species. The pre-germinated seeds were sown in soil media in cell trays, i.e., one seed (for a single-species crop) or two seeds (for mixed species crops) were sown per cell (Fig. 1). For the mixed species crops, seeds of pearl millet and sorghum were relay-planted into cell compartments, containing 1-week-old rice seedlings. Therefore, the mixed-seedlings used in this experiment can be regarded as one of the mixed cropping techniques in the broadest sense. The seedlings of the two different species were in direct contact (Fig. 1), a precondition of close mixed planting. Hence, in this text, we define 'the mixed-seedling' as 'mixed cropping'.

The pre-germinated seeds were sown in Hygromix growing medium (90% v/v peat moss,  $314 \text{ mg NO}_3 \text{ L}^{-1}$ ,  $174 \text{ mg PO}_4 \text{ L}^{-1}$  and  $45 \text{ mg K L}^{-1}$ ; Hygrotech Pty, South Africa). The seedlings were cultivated in 137 L of solution culture, circulating in trays, with each overlying a hydroponic reservoir ( $1.43 \times 0.60 \times 0.30 \text{ m}$ ,  $L \times W \times H$ ), in a greenhouse with natural lighting of approximately 12 h daily and with 56% solar radiation transmittance. A week after sowing the pearl millet and sorghum seeds, the cell trays were cut into individual cell compartments and placed in disposal dishes for the roots to entangle and form root mats (Iijima et al., 2016); thereafter, the seedlings were grown for 2 weeks before being transplanted to field plots. The culture medium was supplemented twice a week with Nitrospray Plus nutrient solution (Hygrotech Pty, South Africa), supplying  $80.3 \text{ mg N L}^{-1}$ ,  $32.8 \text{ mg P L}^{-1}$ ,  $11.0 \text{ mg K L}^{-1}$  and other mineral nutrients. The culture was renewed weekly and the pH was adjusted to 7.4, because the irrigation water was generally alkaline.

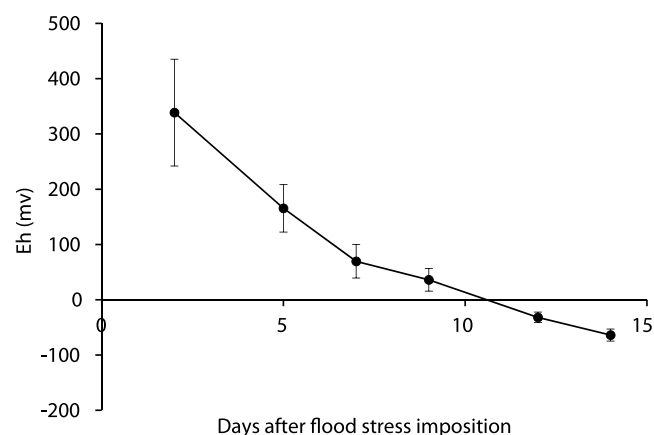
#### 2.4. Field preparation

The fields were ploughed 15–20 cm deep using a tractor-drawn rotary tiller. Following general local recommendations, a basal fertilizer, at the rate of  $30 \text{ kg N ha}^{-1}$ ,  $45 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  and  $30 \text{ kg K}_2\text{O ha}^{-1}$ , was incorporated into the 15-cm soil layer by puddling with a light rotary tiller. After land preparations, the plots for the flood treatments were submerged and seedlings were trans-

planted into the plots. No top dressing was applied during the flooding treatment.

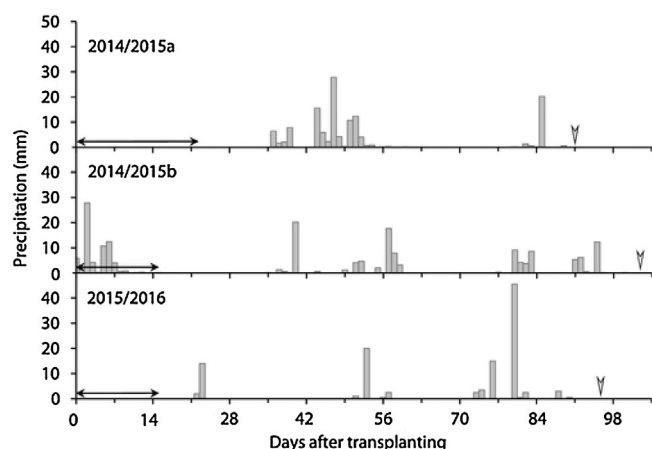
#### 2.5. Cropping system and experimental design

Five cropping systems namely, single-stand pearl millet, single-stand sorghum, single-stand rice, pearl millet mix-planted with rice and sorghum mix-planted with rice were tested in five different experiments to evaluate the mixed cropping concept (Table 1). Four of the experiments were conducted in 2014/2015 to test the survival and production analysis in a randomized complete block design with three replications. In the last experiment performed in 2015/2016 for grain production and analysis of land equivalent ratios, the cropping systems were tested under two flood treatments: non-flooded control (drained soil) and flood treatments, which were arranged in a split-plot design with eight replications, with the flood treatments being the main plots and cropping systems being the sub plots. In all the experiments, 3-week-old pearl



**Fig. 2.** Changes in the soil redox potential during the flood treatment in the 2015/2016 experiment. Vertical bars represent  $\pm$  standard error of the mean ( $n=5$ ).





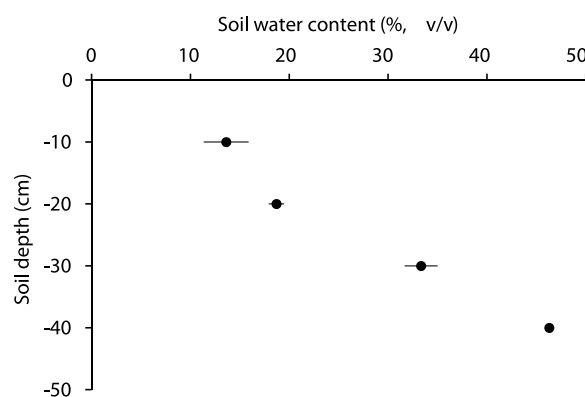
**Fig. 3.** Daily precipitation in the 2014/2015a, b and 2015/2016 production experiments. Horizontal arrows, flood treatment period; arrow head, end of the experiment. Irrigation was done during the dry spell periods.

millet and sorghum and 4-week-old rice seedlings were transplanted into field plots, at a soil depth of 5 cm with a constant spacing of 0.3 m × 0.3 m. In total, 10 and 18 hills per treatment were grown in the 2014/2015 and 2015/2016 experiments, respectively. Mixed cropping treatments had constant plant densities; the plant hills consisted of two plants, i.e. one pearl millet and one rice, and/or one sorghum and one rice but the control treatment had one plant per hill as sown in cell trays. The field size for each of the 2014/2015 experiments was 9 m × 7 m, while that for the 2015/2016 experiment was 32 m × 18 m. NERICA4 was transplanted as a border plant in each experiment. Moreover, before field transplanting compacted earth bands (0.5 m high × 1.0 m wide) were constructed around each experimental plot, and between main plot treatments in the last experiment to separate non-flooded control plots from the flooded treatment plots.

## 2.6. Flood treatments, irrigation management, and yield evaluation

Soil flooding was achieved by applying an amount of about 20–30 mm of irrigation water every one or two days during the flood treatments. In the 2014/2015a experiment, the seedlings were exposed to flood stress for 22 days at a mean water level of 9 cm above the soil surface; while in the other experiments, the seedlings were subjected to flood stress for 11 or 15 days at a mean water level of 5–7 cm. The mean pH value of water in the individual experiments was approximately 7.5; the mean values of soil redox potential (Eh) were –74 mV {7 days after flooding (DAF)}, –183 mV (14 DAF) and –153 mV (9 DAF) for the 2014/2015a, b and d experiments, respectively. In the 2015/2016 experiment, Eh value in waterlogged soil gradually decreased from approximately 338 mV at the beginning of the waterlogged treatment to approximately –64 mV at 14th day of the treatment (Fig. 2). The water pH and Eh were monitored one to three times in the 2014/2015 experiments, and six times in the 2015/2016 experiment, during the flood treatments using a pH (Twin pH, B-211, Horiba, Japan) and Eh metre (PRN-41, Fujiwara Co. Ltd., Japan). Measurements for Eh were taken from 7.5 cm soil depth at the midpoint of the inter- and intra-row spacing, approximately 20 cm away from the adjacent plants.

Following the termination of the flood treatment until crop physiological maturity, plots in the production experiments were given supplemental irrigation during dry spells (Fig. 3). The 2014/2015a experiment was given supplemental irrigation by applying approximately 8 mm at 2–3 day intervals during the dry spells. In the 2014/2015b and 2015/2016 experiments, the



**Fig. 4.** Volumetric soil water content within the top 40 cm soil depth in the 2014/2015a experiment. Horizontal bars represent ± standard error of the mean (n = 7, 7, 5 and 2 at 10, 20, 30 and 40 cm depth, respectively). Measurements were taken one day before each irrigation application.

amount of irrigation water was reduced due to poor dryland crop growth observed in the first experiment; therefore, each experiment received approximately 5 mm of water at 3–4 day intervals during the dry spells. In each experiment, top-dressing was done by band placement during the grain setting stage of pearl millet, about 55–60 days after sowing of pearl millet, at the rate of 3.9 g N m<sup>-2</sup>, 5.9 g P<sub>2</sub>O<sub>5</sub> m<sup>-2</sup> and 3.9 g K<sub>2</sub>O m<sup>-2</sup>. No pesticide was applied during crop growth but insects and weeds were controlled manually. After field draining in the 2014/2015a experiment, soil water potential and volumetric soil water content were measured one day before each irrigation, using a tensiometer (DIK-3162, Rika Kogyo Co., Ltd., Japan) and neutron probe Delta-T PR1 (Delta-T Devices Ltd., Cambridge, UK), respectively. The tensiometer and neutron probe access tube were installed at the midpoint of the inter- and intra-row spacing. Soil water potential measured at 30 cm depth ranged from –14 to –45 kPa. Changes in volumetric soil water content within the 40 cm soil depth are shown in Fig. 4.

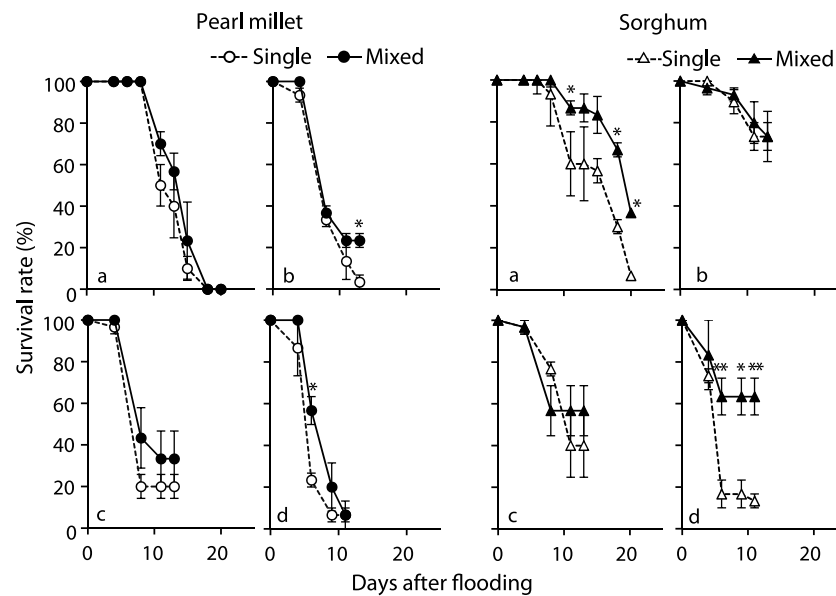
Plants were grown until maturity. Pearl millet was harvested between 90 and 100 days after sowing, while sorghum and rice were harvested between 115 and 132 days. Panicles were air-dried and threshed, and then the clean grains were weighed and the grain moisture content was measured by Grain Moisture Tester (PM-830-2, Kett, Japan). The grain weights were adjusted to 14% moisture content to obtain total yield per plot.

## 2.7. Evaluation of plant survival rates

In the four 2014/2015 experiments, the topmost youngest, fully expanded leaf, was used to determine the survival rates of pearl millet and sorghum during the flood treatments, because in most cases, the selected leaf was the last to turn brownish. In each plot all individual plants were assessed during the flood treatment to determine the extent to which the leaf tissue colour had changed, i.e. from green to brown, relative to total leaf area. The leaf was assigned a percentage score such that 0% represents a healthy green leaf denoting a fully live plant, 50% indicates half brown-half green leaf thus a plant withstanding stress, and 80–100% denotes nearly whole brown leaf area without chlorophylls hence considered as dead. In fact, plants did not recover after this condition (80–100% score).

## 2.8. Mixed crop productivity assessment

Using the grain yields from the 2015/2016 experiment, mixed cropping productivity was evaluated by the land equivalent ratio (LER), which compares yields/ha of monocrop- vs. mixed-species



**Fig. 5.** Survival rates of pearl millet and sorghum mix-cropped with rice (cv. NERICA4) under flood stress conditions at the vegetative growth stage in the 2014/2015 experiments. Plants were subjected to flood stress from the day of transplanting (0 day). Vertical bars represent  $\pm$  standard error of the mean ( $n=3$ ). a–d, 2014/2015a–d experiment (Table 1), respectively. \*\*, and \*, significant difference at  $P<0.01$  and  $P<0.05$ , respectively.

**Table 2**

Grain yields of pearl millet and sorghum, mix-cropped with rice (NERICA4) under flood stress conditions at the vegetative growth stage.

Experimental year	Flooding duration (days)	Treatment	Grain yield ( $\text{g m}^{-2}$ )					
			Pearl millet		Sorghum		Rice	
			Single	Mix	Single	Mix	Single	Mix (Pearl millet)
2014/2015a	22	Flooding	0	0	0	29.9	N/A	233.3
2014/2015b	15	Flooding	78.9	99.2	304.6	360.6	N/A	122
2015/2016	15	Upland	661.7	578.4	431.6	237.2	17.9	5.5
		Flooding	101.2	188.6	129.4	181.7	79.9	54.8
		Two-way ANOVA	**		**			**
		Treatment	n.s.		n.s.			n.s.
		Cropping	n.s.		*			n.s.
		Interaction	n.s.					n.s.

N/A, Rice-single not planted. \*\* and \*, significant difference at  $P<0.01$  and  $P<0.05$ . n.s., not significant.

fields. The LERs for mixtures of pearl millet or sorghum (dryland cereal [d] and rice [r]) were determined following the method described by Mead and Willey (1980):

$$\text{LER} = \text{LER}_d + \text{LER}_r = (Y_{dm}/Y_{ds}) + (Y_{rm}/Y_{rs})$$

where,  $\text{LER}_d$  and  $\text{LER}_r$  represent partial LER of 'd' and 'r' crops, respectively,  $Y_{dm}$  and  $Y_{rm}$  represent the yields of 'd' and 'r' as mixed crops, respectively and  $Y_{ds}$  and  $Y_{rs}$  are the respective yields of 'd' and 'r' as single crops. An LER value of  $>1$  indicates a yield advantage, LER value = 1 indicates no advantage and LER value of  $<1$  indicates a disadvantage for mixed cropping.

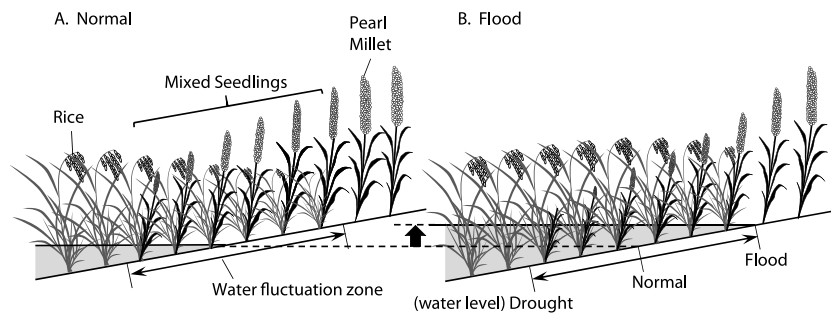
## 2.9. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics, Version 21. For all the 2014/2015 experiments, an arcsine transformation was performed on survival rate percentage data, and single and mixed crop treatment mean values at each observation time were compared by independent samples *t*-test. Moreover, a two-way ANOVA was performed for grain yield data of the 2015/2016 experiment.

## 3. Results

### 3.1. Survival rates of mix-cropped pearl millet and sorghum

The survival rates of flood-stressed pearl millet and sorghum seedlings, grown as single stands and mixed plants with rice, were assessed in the 2014/2015a–d experiments (Fig. 5). The survival rate of pearl millet was generally unaffected by flood stress for nearly 5 days after flooding (DAF), irrespective of the cropping treatments; however, after this period the survival rate tended to decline rapidly though it remained relatively higher in the mixed plants than in the single-stand plants (Figs. 5a–d). At 13 DAF, the survival rates in the single-stand treatments were 40%, 3% and 20% (Fig. 5 Pearl millet a–c, respectively) compared with 57%, 23% and 33% in the corresponding mixed plant treatments. However, in all of these experiments, the survival rate generally dropped fast, and in the 2014/2015a experiment (Fig. 5a), all the plants were killed at 18 DAF. Moreover, the plants in the 2014/2015d experiment (Fig. 5d) remained alive for only about 11 days after flooding, whereas the plants in the other experiments (Fig. 5b and c) were still alive by the 15th day after flooding. With regards to sorghum, plant survival rate patterns were almost similar to pearl millet; in most cases, the survival rate was much higher in the mixed than in the single-stand plants. At 13 DAF, the survival rate of the sorghum mixed plants was 87%, 56% and 63% (Fig. 5 Sorghum a, c, d, respectively) as compared



**Fig. 6.** Possible use of the pearl millet (or sorghum) and rice mixed-seedling system, in flood-affected, rain-fed fields of smallholder farmers in semi-arid regions. This mixed cropping model could provide countermeasures to secure grain production under extreme weather conditions.

**Table 3**

Land equivalent ratio (LER) for mixtures of pearl millet and sorghum mixed-cropped with rice in the 2015/2016 experiment.

Treatment	Pearl millet			Sorghum		
	Partial LER		Total LER	Partial LER		Total LER
	Pearl millet	Rice		Sorghum	Rice	
Upland	0.87	0.31	1.18	0.55	0.18	0.73
Flooding	1.86	0.69	2.55	1.40	0.73	2.14

with 60%, 40% and 13% of their single-stand counterparts. These results indicated that the impact of flood stress was much higher in pearl millet than in sorghum.

### 3.2. Grain production of mixed crops

Table 2 demonstrates the grain yields of pearl millet, sorghum and rice, as influenced by cropping systems and flood conditions. In the 2014/2015a experiment, in which the plants were exposed to 3 weeks flood stress, no yield was obtained from pearl millet because all of the plants were killed by flood stress. However, for sorghum, the mixed plants produced a yield of  $29.9 \text{ g m}^{-2}$ , whereas no yield was obtained from the single-stand plants due to poor filling. In the 2014/2015b experiment with two weeks of flood stress, pearl millet and sorghum in the mixed plant treatments produced 26% and 18% greater yields, respectively, than in the corresponding single-stand treatments. However, the yields of pearl millet were affected by flooding much more than that of sorghum, irrespective of the cropping treatments. In the 2015/16 experiment, the amount of irrigation water was reduced, and rainfall before heading was quite low (Fig. 3), which eventually caused partial grain-filling in the rice. In this experiment, the effects of flooding were significant ( $P < 0.01$ ) on the grain yields of pearl millet, sorghum and rice. Cropping systems did not have a significant ( $P > 0.05$ ) influence on grain production in all the crops. However, the interaction between the flood treatments and cropping systems was significant ( $P < 0.05$ ) on the sorghum grain yields. Overall, flooding decreased the dryland cereal yields, but increased the rice yields. In the pearl millet, under flood conditions, the grain yield in the mixed plant treatment was 86% higher than that obtained from the single-stand treatment. In sorghum, under the non-flooded upland (control) condition, grain yield in the mixed plant treatment was 45% lower than that in the single-stand treatment; conversely, under flood conditions, the grain yield in the mixed plant treatment was 40% higher than that in the single-stand treatment.

### 3.3. Productivity of mixed crops

Table 3 demonstrates the productivity indices for pearl millet–rice and sorghum–rice mixtures, based on the grain yields from the 2015/2016 experiment. The value of the total LER for the

pearl millet–rice system in the flood treatment was 2.55, which was two times higher than that in the non-flooded upland. This value exceeded the minimum value of 1, indicating a mixed cropping yield advantage over single-stand planting in the flood treatment. For the sorghum–rice system, the total LER in the flood treatment was also greater than the minimum value, being 2.14 and again displaying a mixed cropping advantage over single-stand planting for sorghum.

## 4. Discussion

### 4.1. Crop performance

The results of the present study demonstrated that the survival rates of pearl millet and sorghum plants mix-planted with rice, under the different flood treatments, were generally higher than that of their corresponding single-stand plants (Fig. 5a–d). The abrupt fall of all values at about 5 days after the imposition of flood stress indicates the time when the stress symptoms on plants became visible, which was the time when some of the plants were killed by flood stress. Soil flooding reduces tillering, plant height and dry matter in pearl millet (Sharma and Swarup, 1989; Zegada-Lizarazu and Iijima, 2005). Thus, the higher survival rate of the mix-planted pearl millet may be due to a slight root-zone anoxic condition, created by radial  $\text{O}_2$  loss from rice roots into the aqueous rhizosphere. In our previous study,  $\text{O}_2$  concentration of the mixed-seedlings was higher than that of single stand pearl millet under water culture experiment (Fig. 3 of Iijima et al., 2016). The  $\text{O}_2$  released from the rice root system can ameliorate the adverse effects of low  $\text{O}_2$  stress and reduce phytotoxins in the root zone; moreover, it can also facilitate plant nutrient uptake under submerged soil conditions (Armstrong, 1979; Colmer, 2003; Kirk and Kronzucker, 2005). The mixed cropping technique used in the present study was designed to enhance rice and pearl millet root entanglement or rhizosphere interaction, so that the roots of pearl millet would be exposed to the  $\text{O}_2$  being lost radially from the rice roots. Hence, the oxygenated rhizosphere environment possibly caused improved root respiration of the mix-planted pearl millet during flooding, thus allowing the pearl millet plants to sustain growth. The results of this study are consistent with the findings of our previous laboratory study (Iijima et al., 2016). Also, the survived pearl millet tended to form aerial roots from the shoot base, which may help enhance plant survival under the submerged soil environment as these roots can supply  $\text{O}_2$  to root tips to sustain water and nutrient absorption.

In the 2014/2015a experiment, all pearl millet and most sorghum plants in the single-stand treatment were killed by the 3-week flood stress (Fig. 5a), which may be attributed to the higher flood water level and/or longer duration of flooding than the other experiments. Moreover, environmental and/or meteorological conditions seemed to have affected the survival rates of pearl millet and



sorghum. For example, plants in the 2014/2015c experiment, which were associated with a moist environment under higher mean relative humidity (Table 1), had better survival rates than plants in the 2014/2015a experiment (Fig. 5), which were exposed to hot and dry environments due to higher temperatures and lower relative humidity. Although sorghum tends to adapt to short-term flood conditions by forming root aerenchyma (Promkhambut et al., 2011), in this experiment (2014/2015a), grain yield was only realized from the mixed crop treatment (2014/2015a experiment), indicating that the mixed cropping technique was effective. The reduction in the yields of the mix-planted rice across the experiments (2014/2015a,b and 2015/2016) and the increase in the corresponding pearl millet yields (Table 2) seem to reflect the flood stress intensity, as well as rainfall distribution and supplemental irrigation in the respective experiments. High-rainfall flood events of variable duration have recently been experienced in semi-arid regions, such as Namibia (Mendelsohn et al., 2013; Mizuochi et al., 2014), causing yield losses of pearl millet and sorghum (Anthonj et al., 2015). Therefore, the new mixed cropping system of dryland cereals and rice appears to have the potential to prevent complete dryland-cereal grain losses under short-term field flood conditions (Table 2). Moreover, because rice is adapted to the flood conditions, in this cropping system, it is evident that rice would produce some grains when extended or when severe flood occurred and hence can compensate for the dryland-cereal yield losses (Table 2), ultimately, contributing to grain security in such regions.

Productivity analyses demonstrated high total LER values for the pearl millet–rice mixtures relative to single-stand cropping under short-term flood stress conditions (Table 3). The higher LER values indicate that mixed cropping created a yield advantage, which can be ascribed to the flood stress mitigation effects of rice on the dryland cereals. Co-growing plants can improve the microclimate of their neighbours through rhizosphere interaction (Brooker, 2006; Brooker et al., 2015). However, although there is a high land productivity due to mixed cropping effect, it may be important that such an advantage is considered in relation to the absolute yield, to determine whether the LER values represent appreciable grain quantities. For instance, Table 2 demonstrates that the yields of the mix-planted pearl millet subjected to flooding were higher than those of their single-stand counterparts, leading to the high total LER values (Table 3). However, when the absolute yield under the flooding and non-flooded upland conditions were compared (Table 2), the yields under the flood condition were much smaller, indicating that mix-cropping did not substantially increase pearl millet and sorghum grain yields, despite the high LER values. Although no nutrient deficiency symptoms were observed during plant growth, interspecific competition during post-flooding growth may have masked the yield advantage of this cropping technique. Flood stress mitigation by this new cropping technique may be enhanced through judicious agronomic practices, including nutrient management, and through the selection of compatible cultivars that would reduce competition and enhance their complementarity.

#### 4.2. Agronomical implications

Currently, the simultaneous occurrence of both floods and droughts in the same place has become common worldwide. Under such weather conditions, crop cultivation techniques that may accommodate both extremes of water abundance are required to stabilize cereal production. During the process of developing a mixed cropping system of flood-tolerant rice and drought-tolerant grain crops (Iijima, 2011), we found a new possibility in mixed cropping, by enhancement of the flood tolerance of upland crops. The new cultivation model to mitigate flood stress in semi-arid regions could be established by using mixed-seedlings that are

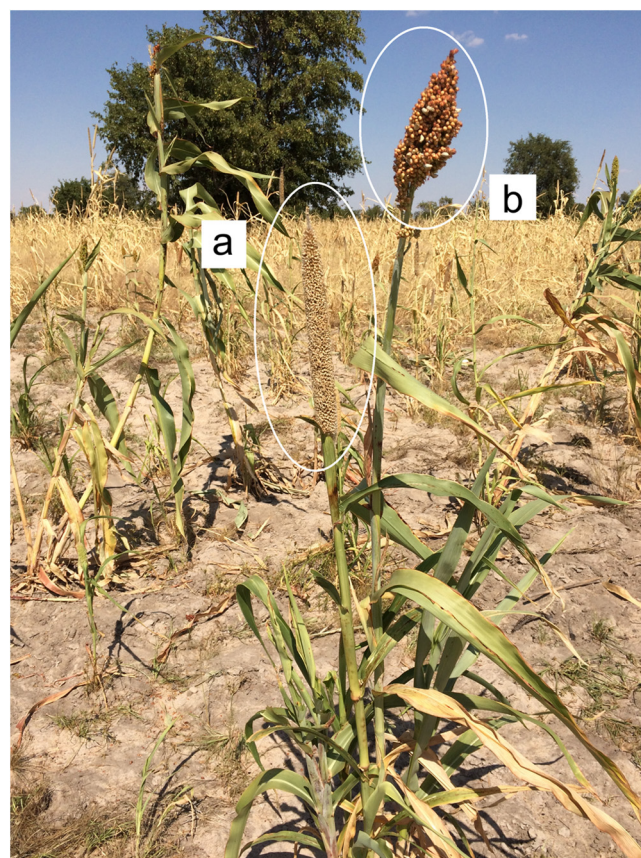


Fig. 7. Mixed plants of pearl millet (a) and sorghum (b) in a farmer's field in Onamundindi village, Namibia, 2015/2016 cropping season.

introduced into the water fluctuation zone of a farm field (Fig. 6). Most of the upland food-crop fields, in resource-poor small-scale holder farms, have small ditches where stagnant water remains after heavy rain or short-term flash floods. The utilization of the wet portions of fields for upland farming would help stabilize food production, particularly in areas with significant rainfall fluctuation. During flooding (Fig. 6B), the survival rate of upland species could be enhanced by using the mixed-seedlings approach, which may contribute to the stable production of traditional drought-resistant cereals by local small-scale holder farmers in semi-arid regions. Furthermore, they could obtain a rice yield by using the mixed cropping system.

In the semi-arid regions of southern Africa, along the borders of Angola with Namibia, Botswana and Zambia, extensive seasonal wetlands are formed during the rainy season by flood water originating from the Angolan highlands (Mendelsohn et al., 2013). A rice-introduction effort (Iijima, 2011; Suzuki et al., 2013) is ongoing in the seasonal wetlands formed in North Central Namibia, a semi-arid region, because the pearl millet fields mostly contain small wetlands where rain-fed, lowland rice can be grown. Flash floods that have become common in this region often adversely affect the harvest of staple food crop, pearl millet. As a result, local farmers often practice mixed cropping of pearl millet and sorghum, which nowadays include mixed sowing within hills (Fig. 7), as a way of ensuring crop security since sorghum is relatively waterlogging tolerant than pearl millet. This is one of the benefits for local farmers when they practically adopted the mixed-seedling concept. However, in the near future, climate change would probably cause more frequent and considerably severe flood in the semi-arid regions. Under such circumstances, the risk of complete crop failure of flood-susceptible cereals may significantly increase. Fur-

ther research on the proposed mixed cropping technique, involving the use of mixed-seedlings (practical model; Fig. 6) could help increase the rice yield in small-scale wetlands and stabilize the yields of traditional drought-resistant staple grains produced by local subsistence farmers. Continuous research and development work are warranted to provide agronomic countermeasures in order to ensure constant staple food production under flood conditions occurring in semi-arid regions because of climate changes.

## 5. Conclusions

Mixed-seedlings of pearl millet and rice or sorghum and rice mitigated the effects of flooding on pearl millet and sorghum. Mixed-seedlings increased survival rates in pearl millet and sorghum, with the impact being much higher in sorghum. Moreover, although grain yields of both the pearl millet and sorghum were decreased by flood stress, in both the single-stand and mixed plant treatments as compared with the yields of the non-flooded upland fields, the yields were mostly higher in the mixed plants than in the single-stand plants under flood conditions. In contrast, the yields of rice were increased by flooding. Furthermore, under flood conditions, the LER values for both pearl millet–rice and sorghum–rice mixtures were >1.0, indicating a mixed planting advantage over single-stand planting.

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